ISCHEMIC MYOCARDIAL METABOLISM AND CELL NECROSIS*

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DURING the last decade we have grown steadily more sophisticated in using biochemical laboratory techniques to evaluate the degree of myocardial ischemia and in our understanding of the pathophysiology of this state. Obviously the therapeutic and diagnostic value of metabolic observations is maximized by a firm understanding of the basic alterations in cell metabolism induced by myocardial ischemia. A lack of familiarity with such mechanisms interferes with an optimal interpretation of data. Under these circumstances an attempt to utilize information obtained from the experimental or clinical laboratory in a given clinical situation may lead to disappointment or confusion. Techniques which should be utilized in many clinical situations may thus be restricted to a few highly specialized centers. Unfortunately there is insufficient time at this conference to describe adequately the entire spectrum of metabolic changes. Several recent summaries present a more thorough review of progress in this area.[1-6]

I should like to discuss briefly some of the distortions of myocardial metabolism induced by ischemia; I shall indicate how some experimental information is obtained and I shall comment on potential pitfalls in interpretation. I shall indicate those factors in the myocardial cellular environment which support viability and help maintain cellular homeostasis, those that most seriously challenge it, and those that offer the possibility of therapeutic intervention. The arrangement of our discussion will also permit me to take note of the more prominent unanswered questions that presently challenge laboratory investigators in this field. Many of the changes to be described are nonspecific; they occur in

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response to other types of cellular trauma and, with some modification, during physiologic cell death as well.

Metabolism implies *living* tissue. The processes of ischemic metabolism are dynamic phenomena that constantly change in degree and severity and are influenced by numerous hemodynamic and biochemical factors. A myocardial infarct rarely presents initially as a homogenous, localized mass of tissue. This type of injury invariably produces a heterogeneous mixture of necrotic and living tissue. This is true of the zone of infarction and also of the ischemic zone which surrounds it.

During the initial 18-20 minutes which follow cessation of nutritional myocardial blood flow all of the changes in this "zone of injury" are reversible. Thereafter, more and more cells suffer irreversible injury, leading to eventual necrosis. Many studies have demonstrated the initial maintenance of normal and ischemic metabolism, occurring side by side, in tissue suddenly deprived of a primary arterial supply. Even after 45 minutes of total hypoxia, 35 to 66% of such cell populations may remain viable.[7]

Significant and measurable changes in carbohydrate, lipid, and protein metabolism and alterations in cytoplasmic, mitochondrial, and lysosomal enzyme systems are demonstrable with the onset of ischemia. These changes establish the basis for several diagnostic approaches to ischemic heart disease and prove useful in evaluating response to treatment. They may be more sensitive than changes seen in the standard electrocardiogram (ECG) and have been shown to precede them. Nevertheless such changes are not in themselves necessarily diagnostic of irreversible cell injury and have not as yet provided a means of quantitating the severity, degree, or extent of the ischemic injury or the size of the infarct. New techniques have improved our ability to perform extensive acute and chronic metabolic studies in patients suffering from myocardial ischemia or infarction. Data accumulated by such studies are of necessity poorly controlled, often limited, and have only recently become available.[8]

Metabolic Laboratory Studies

Our knowledge of the patterns of ischemic myocardial metabolism has been obtained almost exclusively from controlled laboratory experiments performed on the isolated perfused heart of the rat, guinea

pig, and rabbit or on anesthetized open chest dogs. Concepts derived from these studies although individually valid are necessarily derivative. They must be applied with caution to man, in whom humoral and nervous influences and often long-standing coronary artery disease have a profound effect on metabolic response. We cannot expect that all such data will exactly parallel the metabolic events that occur in the ischemic human heart or during the progress of infarction, and we must be prepared to interpret such information carefully.

Isolated systems are necessarily denervated. They are perfused with balanced, well buffered salt solutions, free of hemoglobin and the formed elements of whole blood. Inviariably the partial pressure of oxygen is either much higher or much lower than physiologic levels. Substrate load may be single or multiple but is usually fixed throughout the experiment. Hemodynamic load, and often performance, is pre-set by a carefully regulated perfusion apparatus so that the heart may perform no external cardiac work, may do isovolumic work, or assume a more physiologic pressure volume load. Comparisons between such studies may be confusing since there is an almost linear correlation between substrate utilization-metabolic activity on the one hand and hemodynamic demand on the other. Perfusate solutions may be circulated in either retrograde or antegrade direction. Indeed, coronary flow may be normal or high despite diffuse tissue hypoxia, a state rarely encountered under clinical conditions. The imposed stress of ischemia or hypoxia involves the entire heart by design; pre-existing coronary disease is rarely present. Unless great care is exercised we may easily run afoul of Werner Heisenberg's "Principle of Uncertainty," which points out that the design of the experiment itself may so distort the processes being evaluated as to make the broad interpretation of results highly questionable.

The states of ischemia and hypoxia or anoxia differ significantly in both metabolic and histologic expression. Electron micrographs made from anoxic tissue obtained at the meat market may appear almost normal, while those cut from tissue previously subjected to ischemia show a markedly distorted histology.

Despite rather marked differences in experimental design, data obtained from these studies may not be evaluated critically. The unwary reader may lump findings together and formulate broad, overly simplified general concepts.

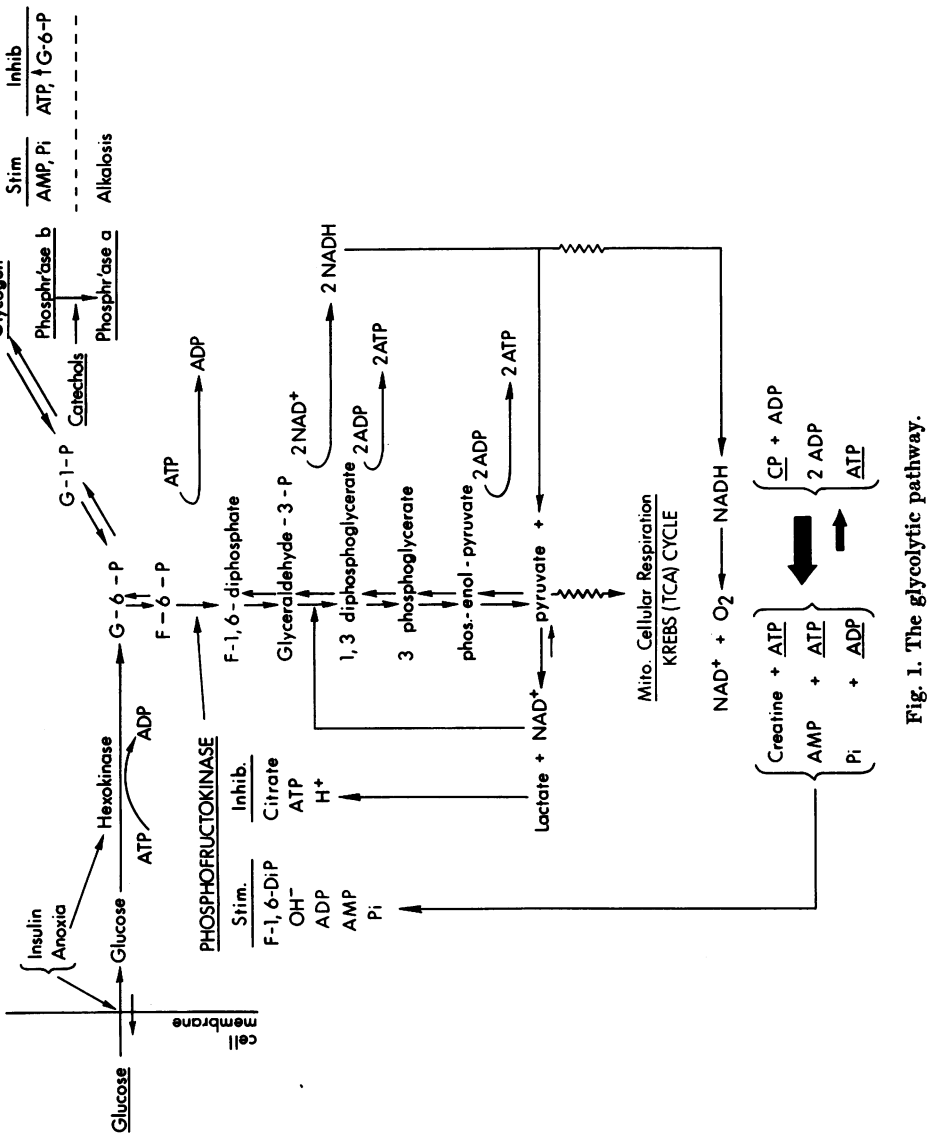

Fig. 1. The glycolytic pathway.

Metabolic Pathways: Aerobic Glycolysis

The initial sequence of reactions in the process of carbohydrate degradation, the so-called Embden-Meyerhof or glycolytic pathway, has been studied in almost all animal preparations. Familiarity with its more general aspects offers constructive insights into ischemic pathophysiology and suggests many potential diagnostic and therapeutic applications.

Figure I illustrates its more salient landmarks. It is a highly integrated self-regulating enzyme system governed by a variety of sensitive allosteric feedback control mechanisms that insure maximal economy in the utilization of substrate. The reactions are extramitochondrial and occur in the cytoplasm of the cell. Several features are of special relevance to this discussion. When coronary blood flow and oxygen content are normal, glucose passes from the extracellular (EC) to the intracellular (IC) compartment, is rapidly and irreversibly phosphorylated in the presence of hexokinase, and in the process utilizes a molecule of adenosine triphosphate (ATP). Glycogen stores are protected by feedback inhibition of phosphorylase due to an increase in the concentration of glucose 6-phosphate (G-6-P). Increased concentration of this hexose monophosphate will also suppress hexokinase activity by feedback inhibition if the end product is not utilized. Its hexose isomer, fructose 6-phosphate (F-6-P), consumes an additional molecule of ATP during a phosphorylation regulated by phosphofructokinase (PFK), a major allosteric enzyme. Although several secondary control points are operative, the sensitivity of this multivalent regulatory enzyme to a number of positive or negative end product modulators has established it as the principal rate limiting pacemaker of the glycolytic sequence. As is true for most enzymes of this type, the cytoplasmic reaction is essentially irreversible.

Cleavage of fructose 1,6-diphosphate yields two three carbon fragments which ultimately produce 4 moles of ATP by cytoplasmic substrate level phosphorylation. Since 2 moles of ATP are hydrolysed, there is a net glycolytic yield of 2 moles of ATP per mole of glucose consumed. An important step in this sequence is the oxidation of glyceraldehyde 3-phosphate to 1,3-diphosphoglycerate, the sole oxidative reaction (dehydrogenation) of the glycolytic pathway. It conserves the energy of oxidation in the end product, the diphosphate, and subsequently releases it to adenosine diphosphate (ADP) by substrate level

phosphorylation. Completion of the reaction is dependent upon the presence of the oxidizing agent NAD^+ (nicotinamide adenine dinucleotide), the obligatory electron acceptor, present in catalytically small amounts. Unless efficient mechanisms for continuous oxidation of NADH are available, feedback inhibition will interrupt the flow of glucose metabolites and terminate the activity of this pathway. During *aerobic* metabolism regeneration is effected by mitochondrial oxidative phosphorylation. A variety of "shuttle" mechanisms serve to transport electrons from extra to intramitochondrial sites. Thus, by the markedly efficient aerobic process of oxidative phosphorylation, the carbohydrate fragments produced by the enzymatic interactions discussed above are ultimately reduced to CO_2 and H_2O after entering the mitochondrion. The net yield of 2 moles of ATP during glycolytic degradation contrasts sharply with the 36 moles of ATP per mole of glucose consumed during mitochondrial metabolism. Thus, to maintain the same energy yield, pure glycolytic energy production would have to consume 18 times as much glucose.

The so-called Pasteur effect, which defines the integration of glycolysis and mitochondrial oxidative phosphorylation, is an expression of the inhibition of glucose consumption and glycolysis during cellular respiration. When mitochondrial oxidative phosphorylation is active, the concentration of creatine phosphate (CP) rises, as does the ATP/ADP ratio. This calls forth negative or inhibitory modulation of PFK activity, and glycolytic flux is markedly reduced. Active mitochondrial production of citrate as a result of the oxidation of free fatty acid (FFA) by the myocardial cell will also induce negative modulation of PFK activity and explains the preferential utilization of FFA during aerobic myocardial metabolism. Increased availability of acetate, pyruvate, and epinephrine will also enhance the concentration of citrate and will depress glycolytic activity.

CP cannot provide an immediately utilizable supply of energy for contractile activity since it is not subject to hydrolysis by actomyosin. It does, however, serve as a readily accessible storage source of energy for regeneration of ATP. The stoichiometry of the reaction in which the enzyme creatine phosphokinase (CPK) catalyzes the reversible transfer of phosphate between CP and ADP markedly favors the formation of ATP. It provides an anaerobic mechanism for the rapid regeneration of ATP.

Anaerobic Glycolysis

When oxygen deprivation is induced experimentally, mitochrondrial respiratory metabolism is compromised and oxidative phosphorylation is no longer capable of maintaining CP or ATP stores. This is reflected immediately and radically by a displacement of the glycolytic reactions outlined above. The rate of glycolysis may increase as much as 15 to 20-fold.[9] Nevertheless, in the anoxic, isolated, perfused rat heart the level of ATP begins to fall within 10 seconds. At 40 seconds after onset 75% of total nucleotides have reached fully reduced levels. Concentrations of ADP, adenosine monophosphate (AMP), and orthophosphate (Pi) rise rapidly to reach a new steady state that exceeds controls by 150 to 200%. Depletion of CP also begins at about 10 seconds, is much greater in magnitude, and falls to half normal levels by 20 seconds.[9] Wollenberger's[10] studies on anoxic canine myocardium showed that changes in the adenylic acid system (ATP, ADP, AMP) were more delayed and were not significant during the first minute of anoxia. He also noted a positive correlation between the rate of increase in the concentration of Pi and the acceleration of glycolysis.

The immediate and marked increase in glycolytic flux induced by anoxia must be supported by an enhancement of glucose consumption, of glycogenolysis, or both. The most severe changes in glycolytic intermediates and enzyme activity are seen during the immediate transition from aerobic to anoxic metabolism. The rapid shift in flux noted during the first two minutes or so has been described as oscillatory,[10] the frequencies lying between 1.5 and two per minute. Oscillations are rapidly damped as a new steady state is established and subsequent variations in tissue levels of intermediates are small.

Morgan et al.[11] have shown in the heart of the rat that anoxia increases the uptake of glucose due to an acceleration of transport, an increase in the phosphorylation capacity, and a decrease in the apparent phosphorylation Km. Reduction in end-product inhibition (G-6-P) of hexokinase by an increase in the concentration of Pi may also enhance phosphorylation of glucose. When insulin was added transport was accelerated still further and near maximal rates of phosphorylation were noted even at very low external concentrations of glucose. In our laboratory[12] we have noted that reduction of flow in the canine heart *in vivo* to 36% of control values induced a fivefold increase in glucose

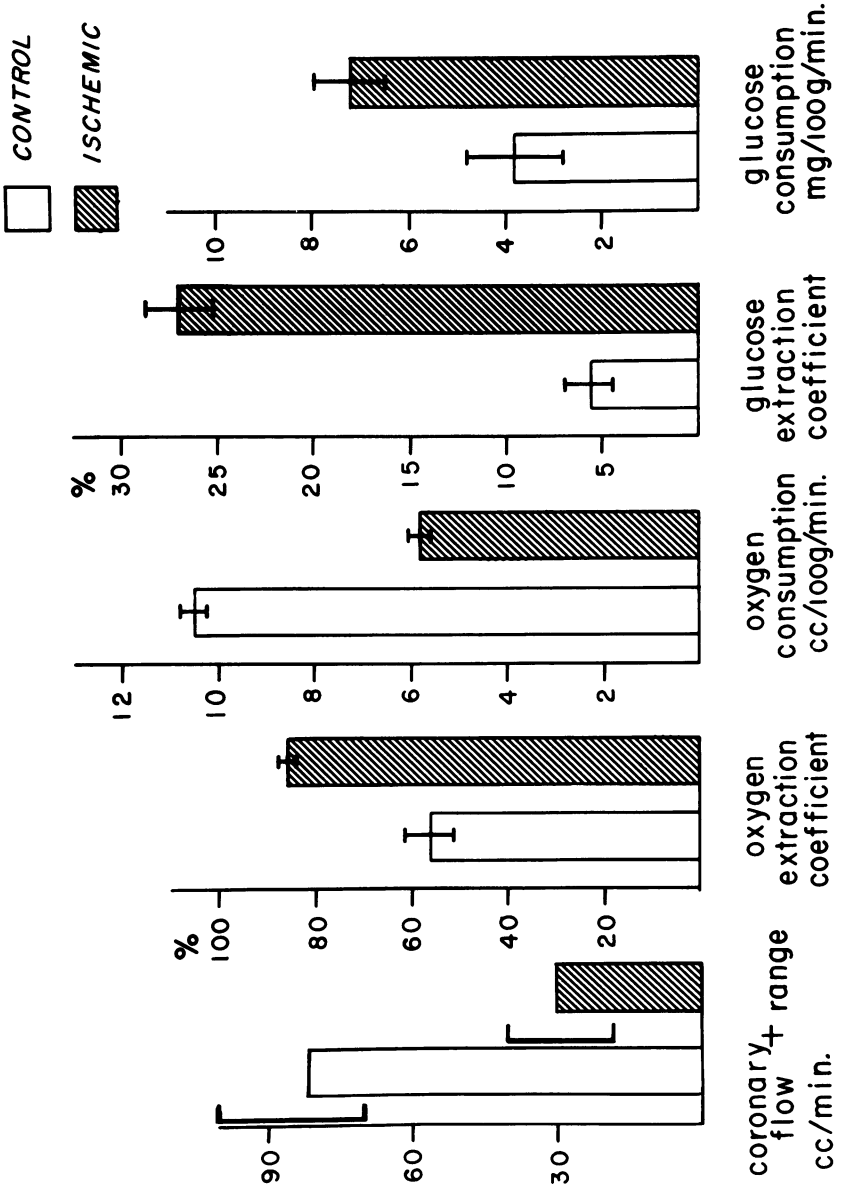

Fig. 2. Effect of myocardial ischemia on oxygen and glucose parameters in the *in situ* dog heart. Left coronary arterial flow reduced to 36% of control.

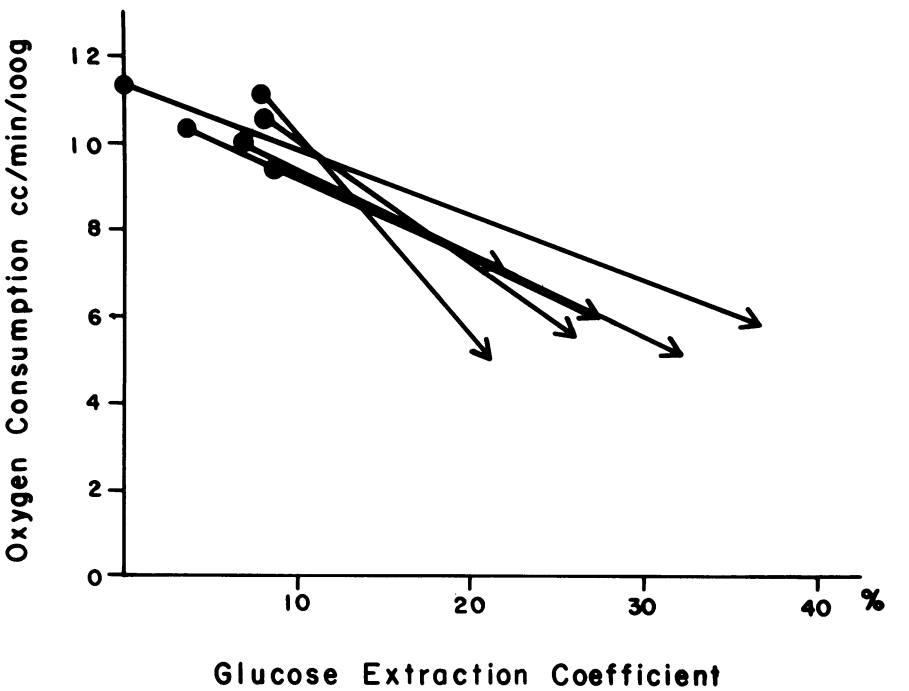

Fig. 3. Correlation of myocardial oxygen consumption and percentage of glucose extracted (glucose extraction coefficient) when left coronary arterial flow is reduced 64% in the canine heart. Reproduced by permission from Brachfeld, N.: Maintenance of cell viability. *Circulation* (Suppl.) *39, 40:* iv-202, 1969.

extraction and doubled the consumption of glucose (Figure 2). Oxygen consumption and glucose extraction coefficient showed a striking, fixed inverse relation (Figure 3).

Glycogen, the endogenous carbohydrate store of the heart, does not normally meet myocardial substrate requirements. In the absence of other sources of oxidizable substrate however, it may account for as much as 25% of myocardial oxygen consumption.[13] The myocardial pool of free glucose supports glycolysis during aerobic perfusion. During severe ischemia this source is unavailable and glycogen provides the sole substrate support for glycolysis. Glycogenolysis is regulated by the enzyme glycogen phosphorylase, normally present almost entirely in the relatively inactive "b" form because of low concentrations of its cofactor, adenosine 5'-monophosphate (5'-AMP), and of one of its

substrates, Pi. Enzyme activity is further repressed by inhibitory aerobic concentrations of ATP, G-6-P, and possibly ADP.[10] When phosphorylated to the "a" form the enzyme is no longer dependent upon 5′-AMP for activity nor is it inhibited by either ATP or G-6-P.[13] With the onset of ischemia or hypoxia the "b" enzyme activity is increased as a function of a fall in ATP and G-6-P concentraton and by subsequent elevations of AMP and Pi, the latter serving as substrate for enzyme action. Of greater importance, however, is the rapid and marked increase in the "b" to "a" transformation mediated by anoxic stimulation of the enzyme phosphorylase "b" kinase. Within 17 seconds phosphorylase "a" concentration rises to nearly 60% of total enzyme concentration. Danforth[14] has suggested that the initial increase in transformation may be related to the slight rise in *p*H which accompanies the early rapid breakdown of CP. The mechanism for maintained activation is disputed. Wollenberger[10] notes the existence of pronethalol sensitive and pronethalol resistant activation of the kinase and suggests that the mechanism is due in part to stimulation of adenyl cyclase and thus of cyclic 3′, 5′-AMP by cardiac catecholamines. Release of catecholamines from postganglionic sympathetic nerves stimulated by ischemia or hypoxia has been demonstrated. Cornblath, however,[15] could not block this conversion by pretreatment with reserpine. Whatever the mechanism, mammalian IC glycogen concentration can be shown to fall to about 70% of control levels within four minutes of the onset of anoxia.

The increase in hexokinase and phosphorylase activity enhances the input of carbohydrate substrate into the glycolytic pathway and shifts integration of the rates of glycolysis and respiration to a new metabolic "set." Inhibition of PFK activity by ATP is most marked at a slightly acidic *p*H.[14] The initial unregenerated breakdown of CP increases intracellular *p*H slightly. IC Pi concentration in the dog may rise from an aerobic value of 2 μmoles per ml. of IC water to between 8 and 16 μmoles per ml. Both factors are operative in initiating PFK activity. The latter thereafter serves a more permissive role since once such activation has begun the reaction tends to be autocatalytic, because of the marked antagonism of fructose diphosphate to ATP and citrate inhibition. Enhanced PFK activity means rapid transport of carbohydrate equivalents down the glycolytic pathway. Mitochondrial oxidative phosphorylation is blocked by inadequate oxygenation, and regen-

eration of NADH is depressed. When aerobic metabolism is operative the isoenzymes of lactic dehydrogenase MH_3 and H_4, with low affinity for pyruvate as an electron acceptor allow NADH to be oxidized readily by mitochondria. With hypoxia the M_4 and M_3H isoenzymes appear to be activated, the affinity of pyruvate as electron acceptor increases, and reoxidation of NADH is accomplished by reduction of pyruvate to lactate. This appears to be a relatively efficient emergency measure. Since lactic acid has no other major metabolic pathway open to it, its accumulation does not interrupt subsequent metabolic reactions. Williamson has shown[9] that lactate levels increase within 20 seconds and continue to accumulate at a rate of 60 to 70 μmoles per gram dry weight per minute until a plateau is reached, indicating that the rate of washout is equal to the rate of production.

Normally, glycolysis is an inefficient and quantitatively insignificant method of energy production. During anoxia or severe hypoxia areas of irreversibly damaged myocardium are certainly incapable of maintaining adequate stores of ATP by such means. The variable sized band of ischemic tissues which surrounds the infarcted myocardium may be extensive and leads a precarious existence balanced between potentially reversible changes and terminal cell death. In this zone, glycolytic mechanisms acquire increased importance and have been shown to meet as much as 30% of mammalian energy requirements. Although there has been much discussion of the potential importance of this pathway of myocardial energy production, the true significance of its contribution to myocardial energetics has yet to be determined. It will certainly not support normal myocardial wall tension and adequate contractility. Nevertheless it can maintain low levels of contractility.[16] Agents that inhibit glycolysis inhibit contractile activity. Work by others[17] and in our laboratory has also demonstrated that the presence of glucose in an oxygen-free perfused heart system will support reduced contractile performance and electrical activity. It will prolong the period of reversible injury and enhance recovery when oxygen is readmitted. There is thus strong evidence that such limited energy production can make a small but perhaps critical contribution to the maintenance of cell viability, to the survival of excitability, and to the function of nodal and conducting tissue until a competent collateral circulation is established or vasodilator tonus is induced. It may also play an important role in promoting the recovery of the acutely anoxic heart.

Myocardial Metabolic Acidosis

Unfortunately, such enhanced glycolytic energy production is not an unmixed blessing. The acidosis that accompanies an accumulation of lactic acid and other reduced metabolites has a deleterious and ultimately lethal action on myocardial function. Williamson[18] has recently demonstrated that the adverse hemodynamic effects of anoxia may be reproduced by a reduction of *p*H even when oxygenation was adequate. Depression in contractility was seen despite normal concentrations of high energy phosphate and may have occurred in response to the active competition of hydrogen ions with calcium at excitation-contraction binding sites. In contrast to other tissue, the mammalian myocardium has a relatively poor intrinsic buffering capacity. Myocardial tissue acidosis is accompanied by a reduction in left ventricular contractility and isovolumic work capacity and by refractoriness to vasoactive and inotropic agents. There is a decrement in cardiac pacemaker activity and an increased susceptibility to dysrhythmias. Coronary flow falls in the face of a decrease in *p*H, accentuating an adverse clinical state, and there may be enhancement of intracapillary blood coagulation. The *p*H optima of tissue enzyme systems is most frequently found to lie within the normal physiologic range. As the *p*H falls such systems are progressively inhibited. A decrease in *p*H to about 6.6 inhibits the activity of the Malate-Aspartate "shuttle," and this primary mechanism for transporting hydrogen ions between cytosomal and mitochondrial pyridine nucleotides is progressively inactivated. PFK is also vulnerable to *p*H inhibition. When anaerobiosis is prolonged or severe, inhibition of PFK activity markedly reduces glycolysis and monophosphate concentrations rise. There is suggestive evidence that at very low levels lysosomal enzymes may be released.[2] Their proteolytic and hydrolytic activity is optimal in the severely acidotic range and may further destroy the biochemical function of the cell and ultimately lead to autolysis.

Diagnostic Implications of Metabolic Changes

The diagnostic and therapeutic implications of these metabolic changes depend upon our ability to sample the venous efflux of the myocardium. Sampling enables us to evaluate change in extraction patterns of common myocardial substrates (glucose, FFA)[8, 19] and can provide valuable clues to the degree of anaerobic metabolism. An

increase in the IC Pi level is accompanied by its appearance in abnormal concentrations in venous blood within two minutes.[20] Potassium ion leakage from the damaged cell not only causes ECG abnormalities but may be reflected by elevations in the coronary sinus samples[21] and is replaced by sodium, chloride, and water. Such electrolyte shifts lead to swelling of cells and depression of contractile activity (see below).

Biochemical Estimation of Myocardial Ischemia

The inability of the tricarboxylic acid (Krebs) cycle pathway to utilize the increased amounts of pyruvate generated by enhanced glycolysis and the accumulation of cytoplasmic NADH forces the conversion of pyruvate to lactate for disposal of H^+ and oxidation of NADH. A reduction in the supply of oxygen to the myocardial cell causes a shift in its oxidation-reduction system to a more reduced state. The oxidized and reduced forms of nicotinamide adenine dinucleotide (NAD, NADH) and the NAD linked dehydrogenase systems immediately reflect this change. Our primary concern, however, is with the NAD-NADH redox potential of the mitochondrion, the site of oxidative phosphorylation and the most sensitive index of the adequacy of oxidation. The intramitochondrial redox state is only indirectly coupled to cytoplasmic NAD-NADH ratios and the latter is grossly reflected by relative shifts in IC lactate/pyruvate (L/P) concentrations.

Such changes in venous L/P concentrations are many stages removed from the critical target site at the mitochondrial level. Venous concentrations represent mixed samples and at best express a net lactate balance. Oxygen deprivation has been qualitatively expressed in terms of a decrease in lactate extraction (% lactate extraction), release of lactate into coronary venous blood samples (lactate production), or as an increase in the L/P ratio of blood perfusing the myocardium.

The validity of this type of estimation requires that the muscle cell membrane be freely permeable to lactate and pyruvate and that there be an equilibrium between cytoplasmic and mitochondrial NADH concentration, assumptions that are not entirely justified. Nevertheless, such data is commonly utilized for diagnostic evaluation of suspected myocardial ischemia. If the clinician is aware of potential pitfalls, misinterpretation may be avoided and this extrapolation considered valid for all practical purposes.[22] Table I lists some of the factors of importance in evaluating myocardial venous lactate data. Although patients with

TABLE I. SIGNIFICANT FACTORS IN EVALUATING MYOCARDIAL LACTATE DATA

1) Nutritional status of the patient:
   a) Infusion of glucose, pyruvate, or lactate. A positive linear correlation between lactate extraction and arterial lactate concentration.
   b) Arterial lactate elevated with shock, hypoxia.
   c) Diabetes.
   d) Serum FFA concentration. May suppress extraction of carbohydrate, induce pyruvate efflux.
2) Alkalosis, hyperventilation, infusion of bicarbonate.
3) Catheter placement, inadequate sampling, segmental disease:
   a) Sampling site may be proximal to vein-draining ischemic area.
   b) Drainage from ischemic areas may be meager or absent.
   c) Dilution of ischemic drainage by normal venous efflux.

myocardial ischemia may show evidence of enhanced glycolysis when coronary reserve is challenged, myocardial glycolysis is accelerated by many factors in the absence of coronary artery disease and despite adequate oxygenation. If the capacity of the hydrogen shuttle is exceeded, lactate may be formed from pyruvate.

Coronary sinus studies should not be performed unless the subject is in a steady (basal) nutritional, metabolic, and hormonal state. Sampling after an overnight fast during a period of active metabolism of elevated FFA is accompanied by suppression of circulating glucose, lactate, and pyruvate concentration, as well as inhibition of pyruvate dehydrogenase activity so that an increased amount of pyruvate is converted to lactate. Glycolysis is also depressed by citrate induced inhibition of PFK activity, extraction of carbohydrate is reduced, and myocardial arteriovenous differences are difficult to determine accurately. Starved rats show a decrease in their myocardial L/P ratio. Conversely, hyperglycemia induced by glucose infusion will enhance its extraction and both pyruvate and lactate production, prevent steady state determinations, and induce a transit time artifact.[23] Lactate and pyruvate infusion may similarly distort data without necessarily reflecting changes in oxygenation. Exercise stress tests, once popular for evaluation of coronary insufficiency, are compromised when utilized for estimation of myocardial glycolysis due to the accompanying increase in arterial lactate concentration and have been replaced by pacing

induced stress. Shock and generalized hypoxia from whatever cause is also associated with marked elevations in arterial lactate concentration and is a contraindication for coronary sinus studies.

Henderson et al.[24] have postulated compartmentation of tissue lactate and demonstrated differing transmembrane concentration gradients for lactate and pyruvate. The rate of pyruvate efflux from the isolated heart was shown to exceed that of lactate by 10-fold, suggesting that a prolonged steady state is required before intracellular-extracellular (IC-EC) equilibration may be assured. Some investigators have therefore abandoned pyruvate determinations and L/P ratios in favor of a lactate assay alone, recognizing that the loss in sensitivity is compensated for by an increased reliability.

The relative inhibition of membrane transport of glucose found in diabetes mellitus reduces glycolytic flux and is associated with a decrease in L/P ratio and with pyruvate accumulation.[22] In studies performed with the isolated diabetic heart, the addition of insulin enhanced glycolytic flow, but pyruvate accumulation persisted. Further, the elevation in circulating FFA and ketone bodies noted in poorly controlled diabetes leads to citrate induced inhibition of PFK activity and further depresses glycolysis. Both FFA and ketone bodies are extracted from the arterial blood in preference to lactate and, when present in significant concentrations, will distort the L/P ratios of the coronary venous samples and cause a misleading drop in the calculated percentage of lactate extraction. Enhanced insulin resistance or latent diabetes, frequently noted in the postcoronary state, must also be considered when such studies are performed.

Other hormones and drugs (growth hormone, cortisol, heparin) modulate glycolytic flux by mobilization of FFA. Perhaps the most common mechanism for the mobilization of FFA from adipose depots is that stimulated by an increase in catecholamine secretion. Patients undergoing diagnostic pacing studies often demonstrate anxiety symptoms and elevated blood catecholamine levels, especially when paced to the onset of angina. Finally it must be noted that glucagon has been shown to accelerate conversion of phosphorylase "b" to "a," thus enhancing glycogenolysis and increasing the output of lactate.

Scheuer[25] reported that alkalosis increased myocardial lactate production and increased exogenous glucose and endogenous glycogen utilization during normal oxygenation. These effects were noted

whether *p*H changes occurred as a result of lowering pCO_2 or by increasing the bicarbonate content. Its mechanism appears to be a stimulation of PFK activity. The Pasteur effect is disrupted by alkalosis as PFK is relieved from ATP inhibition.

During hypoxia, metabolic alkalosis was associated with improved left ventricular pressure and rate of pressure rise, neither oxygen consumption nor lactate production was increased, and L/P ratios were lower than in controls. Huckabee[26] also reported lactate production in hyperventilating human subjects. Thus alkalosis alone may double lactate production.

It is evident that evaluation of lactate data may be inconclusive without simultaneous determination of *p*H, bicarbonate concentration, arteriovenous (A-V) glucose, and FFA levels.

Hexokinase and PFK activity may be accelerated in the isolated heart preparation by an acute increase in work.[27] Glucose and glycogen consumption rises, as does output of lactate and pyruvate. Lactate production is similarly doubled when contractility is enhanced by raising the calcium concentration of perfusate solutions.[28] Other more exotic causes of lactate production in the face of normal oxygenation include cardiac transplantation, uncoupling or interruption of the respiratory chain by cyanide, a variety of idiopathic cardiomyopathies, phenformin induced and idiopathic lactic acidosis, and alcohol ingestion. Alcohol increases cytoplasmic hydrogen ion content at a rate greater than that at which it can be shuttled to the mitochondrion for oxidation.

Gorlin[23] has emphasized that the diffuse and segmental nature of coronary disease often leads to sampling error because of poor or improper placement of the catheter. If the catheter tip lies proximal to the vein draining the ischemic zone, evidence of glycolysis may be missed entirely. The nonuniformity of myocardial blood supply is accentuated during ischemia. Poorly perfused areas may drain a meager volume of blood which is rapidly diluted by mixing with large volume flow from normal zones. Therefore, it is recommended that samples be obtained at three venous sites, from the great cardiac vein to a point just proximal to the coronary sinus.

Despite great care one may still fail to obtain evidence of ischemia by reliance on evidence of anaerobic glycolysis alone. It would seem wise to simultaneously evaluate several parameters during pacing induced stress. Changes in hemodynamics and in the ECG, evidence of

negative potassium and phosphate balance, abnormalities of FFA oxidation and extraction (see below), and oxygen desaturation of coronary sinus samples may supply sufficient supplemental information to support a diagnosis. It is evident that such changes are dependent upon viable though ischemic tissue. Infarcted myocardium is by definition necrotic and devoid of metabolic activity. It will not demonstrate glycolytic activity whatever the stimulus.

Myocardial Ischemia Versus Myocardial Hypoxia-Anoxia

The literature makes a poor distinction between anoxia and ischemic hypoxia. In the former, usually confined to isolated heart preparations, oxygen availability is determined by the pO_2 of perfusate, coronary flow is either normal or increased, and the entire heart is subject to the insult. Ischemic studies, certainly more physiologic, are usually performed on dogs or other large mammals. A localized zone of ischemia is created by ligation, thrombosis, or reduced canulated flow to a significant portion of the left ventricle. Rovetto et al.[29] compared ischemia to anoxia in the perfused rat heart and noted that, although reduced oxygen delivery initially accelerated glycolysis in both preparations, the rate was twice as fast in anoxic as in ischemic hearts because of more rapid utilization of both exogenous glucose and endogenous glycogen. During ischemia EC lactate concentration was 10 times as great as that seen with anoxia. The onset of failure of the preparation correlated closely with the accumulation of tissue lactate. The authors concluded that the greater toxicity of ischemia may well relate to the inability of this preparation to dispose of end products of glycolysis and to prevent the concomitant marked depression of IC *p*H. The earlier onset of acidosis inhibited PFK activity and suppressed glycolytic energy production. Although arterial or perfusate glucose concentration is rarely rate limiting during the rapid flow associated with hypoxia, its concentration may fall precipitously during ischemia. Under these conditions hypoglycemia introduces another significant variable to the utilization of glucose. Somewhat paradoxically, Bing et al.[30] described a similar depression of mechanical performance by acidosis during hypoxia but found that preparations in which *p*H was artificially supported redeveloped only 50% of prehypoxic tension, while the acidotic hearts returned to almost 100% of such levels upon reoxygenation. They propose that the inhibition of glycolysis by aci-

dosis may conserve carbohydrate reserves and delay deterioration during acute, short-lived hypoxia and thus facilitate recovery upon reoxygenation.

Therapeutic Approaches

The administration of a therapeutic agent requires a means of reaching the ischemic cell. Unfortunately, most therapeutic approaches are hedged by the inability of drugs, oxygen, substrates, etc. to reach the site of injury because of the very hemodynamic conditions that initiate the process. Nevertheless, the defects reviewed above suggest several new approaches to the treatment of myocardial ischemia.

Myocardial Cell Swelling in Response to Ischemia

Many workers have noted the persistence of myocardial ischemia and reduced tissue perfusion following restoration of normal proximal flow in the experimental animal. Similar failure of "reflow" to return to normal has been noted in the brain and kidneys.[31] When inadequate oxygenation is prolonged or severe, cell swelling induced by loss of potassium and IC accumulation of sodium, chloride, and water may be accentuated by the addition of osmotically active particles contributed by autolytic breakdown of protein and tissue metabolites. Myocardial swelling may lead to a palpable firmness of the ventricular wall; pliability is reduced by high membrane tensions and contractility is depressed. IC calcium ion concentration is diluted and the normal inotropic response to this ion, already burdened by competition with H^+ ion for contractile binding sites, is lessened. Swelling of capillary endothelial cells may cause trapping of formed blood elements, further reducing flow and inducing a "secondary" type of ischemia which may potentiate the initial hypoxic trauma. Thus, ischemia may beget further ischemia and impede both substrate and oxygen flux.

An obligatory EC hyperosmolal agent, mannitol, improves reflow and prevents closure of regional renal and cerebral vascular beds after temporary arterial occlusion. This therapeutic modality is presently being utilized clinically. Willerson et al.[32] infused this agent into the aortic roots of dogs before and during reversible ischemia of the left ventricle. They reported that an increase in serum osmolality reversed the depressed ventricular function curve, lessened ST segment elevation, and raised total and collateral coronary blood flow. The improved

postischemic myocardial performance persisted after the return of osmolality to control levels. The mechanism of action of hypertonic mannitol is not entirely clear, but these reports suggest that it prevents closure of capillary beds and decreases cell size, thus enhancing relative IC calcium concentration. Studies in our own laboratory support this hypothesis.[33] We have been unable to demonstrate enhancement in energy metabolism or total flow but recovery of hemodynamic parameters was significantly improved following reoxygenation when mannitol was present. The effect of hypertonic mannitol was related to a direct myocardial osmotic action. The increase in tissue water content expected with prolonged ischemia was less marked and the wet weight/dry weight ratio fell from 7.55 to 6.77 in its presence. Further effects may be mediated by the known ability of hyperosmolality to alter both contractile element and series elastic mechanical characteristics.[34] Unfortunately, the clinical applicability of an agent that expands IC fluid volume during or following an episode of myocardial ischemia has not yet been determined.

Glucose-Potassium-Insulin (GIK) Infusions

In 1939 Selye[35] reported that myocardial resistance to cardiotoxic agents was reduced by IC hypokalemia and hypomagnesemia. Resistance was regained when this ionic imbalance was restored. More recently Sodi-Pallares et al.,[36] Larcan,[37] and others described a regimen for the early treatment of myocardial ischemia which consists of dietary regulation of sodium, potassium, and water intake, and an intravenous (IV) infusion of 10% glucose containing 40 mEq. of KCL, 20 units of regular insulin, and 10 mg. of heparin (Sodi-Pallares) or dibasic potassium phosphate, K^+ and Mg^{++} aspartate, cocarboxylase, and cytochrome-C (Larcan). Treatment was reported to decrease the frequency, duration, and severity of angina pectoris, dysrhythmias, congestive heart failure, fever, and shock. ECG changes rapidly normalized and disturbances in IC water, sodium, and potassium balance were corrected. Response to therapy was attributed to a restoration of IC potassium concentration mediated by insulin stimulated glucose transport and enhanced glycolysis.

The impatient advocacy of its proponents provided an enthusiastic and hopeful reception to these "polarizing solutions" despite the lack of convincing rationale for their use. Earlier we noted that glycolytic

activity is near maximal in the insulinized cell during normoglycemia and that high rates of flux have been demonstrated at low external glucose concentrations.

Admittedly, a low arterial threshold is an artifact of isolated systems not seen *in vivo*. Nevertheless, under all but the most severe degrees of hypoglycemia, physiologic arterial glucose concentrations are adequate and do not limit glucose transport.

GIK infusions are in common clinical use in many hospitals here and in Europe. Unfortunately, clinical trials of this regimen were negated by variations in criteria for the selection of patients. Data describing administration, metabolic, hemodynamic, and electrocardiographic controls were incomplete; and concurrent therapy and subgroup analysis was either inadequate or differed markedly within or between studies. A large multicenter clinical trial of 13 hospitals and 986 patients conducted by the Research Council of Great Britain[38] is typical of those reported; it failed to demonstrate a significant difference in mean mortality between control and treated groups. Mittra[39] reported one of the few large, affirmative clinical studies. There was "spectacular improvement" in 370 trial and post-trial evaluations. The control group mortality of 41% in patients over 60 years of age was significantly higher, however, than that reported by most investigators. A recent experimental study by Maroko[40] indicates that cell viability and contractile potential appear to show a favorable response to infusions of GIK or hypertonic glucose when administered after ligation of the anterior descending coronary artery of the dog. It seems doubtful that stimulation of anaerobic glycolysis is responsible for these findings. If one makes the disputable assumption that systemically administered GIK actually does reach the ischemic zone, it is difficult to understand why such solutions have proven ineffective when given by direct intracoronary infusion.

Each component of the solution has rather wide ranging metabolic effects. When given in combination their actual locus of action is difficult to determine. The rapid introduction of 10 to 20% glucose may quickly depress circulating potassium concentration for as long as six to nine hours and be severe enough to induce changes in the ECG. There is a simultaneous and quantitatively similar IC movement of inorganic phosphate. Experimental administration of Pi to intact cells has been shown to stimulate glycolysis and glycogenolysis and suggests a

possible mechanism of action. Other studies indicate that glucose in high concentration may have a direct effect on transmembrane action potential. Insulin has a multivalent effect on cell metabolism and may play a direct and independent role in modulating myocardial ion transport. It can enhance inorganic phosphate uptake, accompanied by IC migration of potassium, and hyperpolarize the muscle cell membrane. Insulin may also directly stimulate contractility independent of glucose transport or of the anoxic state.

The histologic lesions associated with well oxygenated but hypokalemic cells are quite distinct in appearance from those seen after ischemia or anoxia. Reduction in the transcellular potassium gradient by intracoronary infusion of potassium chloride can reproduce the ischemic sequence of injury potential, ectopic beats, and tachycardia despite normal coronary blood flow.[41] Thus, electrocardiographic changes reflect shifts in IC-EC potassium concentration and do not necessarily indicate myocardial redox state.

It is difficult to assess the role of potassium in ischemia. Egress of potassium is a response to ischemia, not the cause of the biochemical lesion. Its leakage from injured cells induces membrane hypopolarization and a fall in transmembrane resting potential which leads to shifts in the ST segment of the ECG as well as to the onset and potentiation of ectopic foci. Although we may assume that a negative myocardial A-V potassium difference is due to efflux of potassium from ischemic cells, we have no experimental proof that these same injured cells regain potassium when positive balance is restored. The response of the ECG to potassium oriented therapy may be caused by changes in IC-EC potassium ion gradients of the normal or near normal cells in the heterogenous zone of ischemia, rather than by changes in ischemic or necrotic cells.

Reflow experiments suggest that the presence of glucose or potassium is less important than the volume of fluid infused and its osmolal potential. Hyperosmolal solutions may enhance reflow, reduce high membrane tensions, and improve compliance. It has also been shown that the antidysrhythmic effect of GIK is apparent only by IV infusion.[42] The constituents of the solution appeared to be less important than their rate of infusion and osmolality. Hypertonic glucose, saline, and sucrose were equally effective in the treatment of dysrythmias induced by ischemia and in the reduction of potassium efflux.

Role of Glycogen

The *in situ* heart normally shows little or no dependence upon glycogen as an oxidizable substrate. Preferential utilization of exogenous carbohydrate, FFA, and ketones has been demonstrated by many authors. Unlike liver and skeletal muscle, fasting stimulates myocardial glycogen synthesis mediated by fatty acid mobilization, citrate induced inhibition of PFK, and increased levels of G-6-P. When the isolated heart is perfused with substrate-free buffer, glycogenolysis follows utilization of endogenous triglyceride and may account for 15 to 20% of oxygen consumption.[13] The acceleration of glycogenolysis noted after an acute increase in heart work, despite the presence of exogenous glucose, appears to be a linear function of Pi concentration. This hypothesis is supported by experiments which have demonstrated a rapid temporary fall in ATP and CP values and increase in ADP and Pi content under these circumstances.[13, 43] Glycogen utilization is again mediated by an increase in PFK activity, a reduction of G-6-P concentration, direct stimulation by Pi, and release of phosphorylase inhibition. Thus, glycogen utilization in the aerobic heart is regulated by the availability of alternate substrate and by the magnitude of the work load.

During anoxia glycogen plays a much more active role. When the beating isolated rat heart is subjected to an acute work load, the initial peak rates of lactate formation are due to glycogen breakdown. Stores are rapidly depleted.[44]

Glycogen is found in highest concentration in the conduction tissue and ventricular endocardium, areas particularly sensitive to inadequate oxygenation. This distribution offers a teleological emphasis to its importance as an emergency fuel. It is 30% more efficient as a carbohydrate substrate than is glucose and has a net glycolytic yield of 3 moles of ATP per mole of glucose equivalent consumed. It is distributed within the cell as a labile fraction, subject to relatively rapid accumulation and depletion, and a more stable residual fraction. Scheuer[45] enhanced glycogen stores in the rat heart by pretreatment with reserpine and investigated its potential protective role when hearts were subsequently perfused with anoxic buffer. Those taken from reserpine treated rats had higher left ventricular peak pressure and lactate output after two minutes of anoxia than did controls. The rate of glycogenolysis correlated well with the initial glycogen concentration and proportion-

ately more lactate was produced from glycogen than from glucose. Anaerobic ATP production per mole of hexose consumed improved significantly. Both minor and marked elevations in cardiac glycogen stores improved glycolytic reserve and mechanical resistance to anoxia. Similar observations were made in the intact anoxic working canine heart by Hewitt et al.[46] Glycogen content was elevated by prefeeding with butcher fat and water and anoxia was induced by 95% nitrogen ventilation. During a five minute period glycogen utilization in the fat fed dogs exceeded that of controls by 141%. The hemodynamic parameters noted above as well as cardiac output and left ventricular work significantly exceeded controls. In both studies the degree of glycogen utilization correlated well with the level of stored glycogen and suggested that high levels favor increased glycogenolysis during anaerobic metabolism.

Data obtained from working mammalian perfused hearts indicate a maximal glycolytic flux (glucose + glycogen) of 6.2 times the Langendorff control when heart work is acutely increased and insulin is present in the buffer.[44] This maximal value of 3.5 μmoles of glucose utilized per min./gm. wet weight is significantly less than the maximal capacity of phosphofructokinase of 42 to 60 μmoles/min. The increase induced by alkalosis and by anoxia was found to be a third as great. Scheuer also noted the failure of hypoxia to stimulate the mammalian heart to its fullest glycolytic potential. If the rates achieved during his anoxic studies had been reached during mild hypoxia, ATP production would have been theoretically capable of supporting mechanical activity. The observation that the rate of glycogenolysis falls long before glycogen is depleted continues to puzzle investigators.[15] It may well be that only "labile" glycogen can be rapidly mobilized. Inhibition of glycolysis by the attendant acidosis is only part of the story since such inhibition would be present to an even greater degree during anoxia, a finding that is incompatible with the observations noted above. Unfortunately, all the factors that limit mammalian glycolytic rate to submaximal levels remain unknown.

In contrast, the more primitive reptilian heart working in a totally anoxic environment demonstrates a unique anaerobic metabolic capacity. Despite PFK tissue activity and maximal rates of lactate production similar to the mammal, it can meet total energy requirements by anaerobic glycolysis and produce energy in quantities sufficient to maintain via-

TABLE II. COMPARISON OF (U-^{14}C) GLUCOSE UTILIZATION BY ISOLATED PERFUSED TURTLE AND RAT HEARTS IN THE AEROBIC AND HYPOXIC STATES

	Heart rate beats/min.	*Glucose uptake (μmoles/gm. wet wt.)*	*$^{14}CO_2$ (μmoles/gm. wet wt.)*	*Lactate production (μmoles/gm. wet wt.)*
Aerobic				
Rat (A)	280 ± 10	26.8 ± 2.8	2.52 ± 0.27	48.4 ± 10.0
Turtle (B)	39 ± 3	4.8 ± 0.2	0.70 ± 0.07	4.9 ± 0.9
Hypoxic				
Rat (C)	113 ± 7	119.3 ± 7.6	1.69 ± 0.12	223.2 ± 10.8
Turtle (D)	34 ± 3	6.3 ± 0.61	0.20 ± 0.03	36.4 ± 4.5
% Change				
A-C	-60	+345	-33	+361
B-D	-13	+ 31	-71	+642

All values are means ± SE expressed per 30 minutes of perfusion to permit comparison between rat and turtle heart experiments. (U-^{14}C) glucose and palmitate were present as substrate. (U-^{14}C)-glucose concentration was 8.4mM. in turtle and 10mM. in rat experiments. Palmitate concentration was 0.4mM. in turtle and 0.5mM. in rat experiments. N = 6 for each series of experiments.

bility and support contractile, electrical, and metabolic activity at near normal levels. It does so by the utilization of glycolytic enzymatic pathways at maximal efficiency.

Studies of aerobic and anoxic metabolism of the turtle heart (*Pseudemys Scripta*) performed in our laboratory[47] demonstrated a remarkable adaptability to changes in its IC oxygen milieu. The reduced cardiac work of the turtle heart is matched by quantitative differences in substrate utilization. Divergence in metabolic demand between these species may be corrected when data is expressed as units/gm. wet wt./beat (Table II). During aerobic perfusion the turtle heart preferentially extracted and oxidized FFA despite the presence of available glucose. FFA and glucose metabolism was similar to that seen in the mammalian heart and was adequate for myocardial energy requirements. When hypoxia supervened these similarities in substrate extraction and utilization were disrupted. In the rat an enhanced glycolytic flux was met by an increase in exogenous glucose extraction. Glucose uptake per beat exceeded that of the turtle heart by 10-fold. In contrast, the turtle maintains an endogenous glycogen content 10 times that of the rat heart. It provides a readily available source of metabolizable carbohy-

drate, and glucose uptake/beat increased insignificantly. The ability of FFA to depress glucose uptake and oxidation in the mammalian heart was not apparent in studies of turtle myocardial metabolism during oxygen deprivation. FFA utilization did not compete with but rather enhanced concomitant carbohydrate metabolism and glycogenolysis and helped to provide sufficient substrate to meet myocardial energy requirements with little change in hemodynamic performance. The concentration of residual glycogen was adequate to maintain stores and utilization of exogenous glucose was strictly limited. During prolonged perfusions (two hours or more) such stores were eventually depleted, exogenous glucose supplied a proportionately greater amount of oxidizable carbohydrate and its metabolic pattern more closely resembled the mammalian. A large store of labile glycogen appears to be the basis for this advantageous metabolic flexibility. It permits normal function under environmental conditions that are lethal to the mammal. In turtle myocardium a relatively low concentration of hexokinase and enhanced glycogen phosphorylase activity combine to support this pattern.

Despite the marked increase in lactate production during active myocardial glycolysis in the turtle the extremely high quantities of reptilian body buffer help to support tissue *p*H. Robin et al.[48] investigated the effects of 100% nitrogen inhalation and NaCN administration on blood gases, acid-base, and L/P metabolism in the turtle. He noted a slightly alkalotic control blood *p*H which remained in the alkalotic physiologic range for as long as three hours after onset of anaerobiosis. Much of the production of CO_2 resulted from bicarbonate buffering of hydrogen equivalents produced by anaerobic metabolism. He also pointed out that turtles possess a large volume of coelomic fluid, with a *p*H more alkaline than plasma and a bicarbonate concentration approximately three times that of plasma. Further, it has been shown that the sodium "pump" of the turtle bladder, and perhaps of other tissue, may operate for long periods in the absence of oxygen. Maintenance of cellular *p*H thus helps prevent the depression of glycolysis associated with IC acidosis in the mammal.

Free Fatty Acid Metabolism

Plasma FFA appears to be the major myocardial substrate in the postabsorptive state of normal man. Their oxidation accounts for more than half of the oxygen consumption of the heart.[49] In the aerobic state

FFA have been shown to increase myocardial oxygen consumption, preserve or enhance myocardial glycogen stores, and depress glucose uptake. Unfortunately little is known about the relative effects of FFA and carbohydrate on myocardial hemodynamic performance. The general agreement regarding the importance of carbohydrate to ischemic metabolism is not shared when FFA metabolism is reviewed. Some workers[50-52] report that elevated FFA concentrations increase myocardial oxygen uptake excessively, increase the frequency of dysrhythmias, and depress contractility in the hypoxic heart or papillary muscle. Others[53-55] have been unable to confirm these observations. When palmitate + glucose was added to the moderately hypoxic rat heart perfusates in our laboratory[55] normal carbohydrate-FFA relationships persisted. There was a significant depression of carbohydrate utilization and of lactate production. During aerobic perfusion palmitate depressed glycogenolysis and moderately inhibited glucose uptake. When pO_2 was reduced, glucose uptake fell but glycogenolysis remained essentially unchanged with glucose and palmitate perfusate. These findings only seem incompatible with our earlier discussion of enhanced glucose extraction and lactate production during *marked* anaerobiosis when we fail to consider the level of oxygenation provided the cell. The presence of palmitate increased peak systolic aortic pressure over controls and did not disturb heart rate, rhythm, or cardiac output at either high or low oxygen levels. Utilization of FFA may support hemodynamic performance by reducing the severity or delaying the onset of tissue acidosis induced by glycolysis or by supplying oxidizable substrate to tissue whose glycolytic potential has been reduced by a fall in intracellular *p*H. The ATP yield (moles ATP/gm. substrate consumed) of the complete oxidation of palmitate exceeds that of glucose by two and one half times and makes it an advantageous fuel when oxygen supply is limited. Regan[53] also noted a fall in glucose uptake and lactate production when FFA levels were moderately elevated in the ischemic canine myocardium by small doses of norepinephrine. These and other studies[54] of rat, dog, and turtle[47] myocardium have reported a persistent uptake and oxidation of FFA during ischemia. Suppression of carbohydrate metabolism by FFA must therefore persist to some degree despite reduction in available oxygen and correlates with *relative* cellular pO_2. Ischemic FFA metabolic patterns are incompletely understood and reports of extraction, oxidation, esterification, and storage

as neutral lipid are often contradictory. It is not possible to state whether FFA are "good" or "bad" for the ischemic myocardium since ischemia cannot be regarded as an all-or-none phenomenon. Myocardial cells within this area reflect an entire spectrum of oxygenation and metabolic rates. Biochemical determinations present a mean response at best. In general, FFA uptake appears to be fixed or increased during ischemia.[53, 56] The efficiency of cellular oxidative phosphorylation determines whether extracted FFA is oxidized or esterified. Storage as neutral lipid, to be expected during extreme anaerobiosis, cannot occur unless a carbohydrate precursor is available to provide the α-glycerophosphate skeleton required for esterification.[56, 57]

Reports of a reduction in mechanical myocardial efficiency when FFA was offered as substrate during ischemia[50, 51] suggest an uncoupling of oxidative phosphorylation or direct cellular toxicity. There is inadequate documentation to warrant application of these observations to humans, however. In many reports FFA/albumin ratios were abnormally elevated due to the type or amount of FFA used or to relative albumin concentration. In some studies FFA was the sole substrate offered. Unbound FFA in even moderate concentration is indeed toxic to the cell but this is an artificial, laboratory induced state not seen clinically. Opie[54] points out that an increase in oxygen uptake is not seen when a physiologic concentration of FFA or a perfusate containing mixed substrate is used. Conflicting conclusions regarding the stimulation of dysrhythmias in ischemic canine hearts due to elevation of circulating FFA are undoubtedly due to differences in the preparations studied. Elevation of FFA by administration of heparin has not increased the incidence of serious dysrhythmias in patients with recent myocardial infarction.[54]

Changes in FFA metabolism may be extremely sensitive to alterations in oxygen availability and may be utilized as a diagnostic aid in cases of atypical angina pectoris. Patients with normal nutritional blood flow show an enhancement of the myocardial fractional extraction of FFA during a pacing induced stress. In those with pacing induced myocardial ischemia there is a reversal of the normal pattern.[58] Extraction falls but oxidation of the FFA transported into the cell is significantly increased, a finding that is compatible with our discussion.

A unique experimental approach to the problem of increasing anoxic energy production has been suggested by Penney and Cascarano.[59] They

attempted to increase substrate level mitochondrial synthesis of ATP by reversed electron flow. Rat hearts were perfused with mixtures of glucose and various Krebs cycle mitochondrial metabolites. Solutions containing 20 mM. glucose + malate + glutamate and 20 mM. glucose + oxaloacetate + α-oxoglutarate were evaluated during anoxia. A significant increase in heart rate and glucose uptake was noted with both mixtures. ATP concentration increased during perfusion of the former and both glycogen and CP with the latter. Results were inconstant and the mechanism of action obscure. Oxidation of glucose appeared to be essential. The solutions were markedly hypertonic and contained higher levels of metabolic intermediates that that which could reasonably be achieved *in vivo*.

A recent report by DeWall et al.[60] described the IV administration of Allopurinol to sheep and dogs following coronary ligation. This xanthine oxidase inhibitor was said to improve contractility, hemodynamic performance, and electrocardiographic changes of ischemia. There was a decrease in Pi leakage and a reduction in ischemic induced dysrhythmias. The authors suggest that the drug acts to maintain the total body pool of functional purine bases, preserving them for reformation of high energy nucleotides when oxygenation improves.

Therapeutic Conclusions

Prophylaxis must remain our principal approach to the problem of coronary insufficiency. Although I do not have to deal with this thorny problem I must nevertheless emphasize that whatever drug, nutritional regimen, or hormonal intervention is utilized therapeutically, an effective collateral flow is required for delivery of treatment to the target site, the ischemic cell. The struggle is to match energy demand with supply. Metabolic defects must be corrected. Hyperthyroidism and adrenocortical insufficiency increases oxygen and energy demand and lowers cardiac glycogen concentration. Diabetes mellitus inhibits glucose transport and suppresses glycolytic flux. Although hyperglycemia cannot enhance energy production, hypoglycemia is decidedly toxic to the oxygen depleted cell and is to be avoided. Methods to enhance glycolytic flux are being developed. The factors that prevent hypoxia from stimulating myocardial glycolysis to its maximum potential are unknown. Further study of our reptilian ancestors may yet provide the secret of more efficient utilization of this pathway. Techniques for

counteracting the detrimental effects of acidosis on glycolysis and hemodynamics by manipulation of nontoxic buffers are to be encouraged. Alkalotic therapy has proven essential to successful cardiac resuscitation. Although the rate of fall of *p*H due to lactate accumulation is about 0.5 *p*H units/15 min., phosphofructokinase activity and contractile sites are quite sensitive to slight changes in H^+ ion concentration. An elevation of *p*H may increase glycolytic flux, enhance excitation-contraction coupling, and increase coronary blood flow. The enhanced performance of hypoxic hearts rendered alkalotic confirms its protective role. This treatment is still experimental and may have profoundly deleterious effects on cardiac function if accompanied by changes in pCO_2. The cell swelling which accompanies the distortions in ion flux of prolonged cellular hypoxia has been challenged by a most provocative therapeutic approach, that of induced EC hyperosmolality. Its application to the ischemic human heart is awaited. The experimental use of GIK solutions, hyaluronidase, hydrocortisone, and methylprednisolone have all been reported to reduce ischemic damage to the myocardial cell. Their clinical usefulness will undoubtedly be evaluated objectively in the near future. The protective value of increased cardiac glycogen stores prior to the onset of ischemia has been noted in several experimental studies in reptiles and mammals but has not been confirmed clinically. Effective means for elevating myocardial glycogen concentration are cumbersome or may be contraindicated; they include the use of reserpine or β-receptor blocking agents to deplete or interrupt catecholamine stimulation of phosphorylase activity, administration of growth hormone, citrate, ketones, lipids, starvation, or potentially toxic pharmacological agents. It is encouraging to note that physical training appears to have a pronounced positive effect on myocardial glycogen concentration. Techniques that carefully control circulating FFA concentrations are confined to the experimental laboratory. Initial reports indicate sufficient potential to warrant further investigation. Methods that can effectively increase the energy output of the ischemic cell have yet to be developed. Regardless of how it may be boosted, glycolytic energy production cannot conceivably meet the total energy demands of the normally beating cell. The heterogeneous nature of myocardial ischemia, however, does not require that it do so. If we can accept a decrease in contractility of less than 50% of normal for these cells, anaerobic metabolism is capable of maintaining viability.

Present clinical efforts are therefore more pragmatically directed toward reducing the work load of the ischemic myocardium in an effort to meet the deficit and more equitably balance energy demand and supply. The use of β-blocking drugs to reduce heart rate and contractility and furosamide and Arfonad to reduce preload and afterload, respectively, are currently being evaluated at many centers.

I conclude by posing several provocative questions that remain unanswered.

1) Can absolute flow be enhanced to an ischemic zone?

2) What is the optimum substrate mixture for the ischemic myocardial cell? Are FFA beneficial or hazardous?

3) How can we easily increase myocardial glycogen stores in the preinfarct state?

4) How can we quantitate the severity of IC hypoxia or acidosis?

5) What specific biochemical or anatomical changes limit reversibility in myocardial ischemia?

6) Why has ischemia proved to be a more serious threat to survival of viability (biochemical, histopathological) than anoxia? Is it because of the suppression of glycolysis by severe acidosis?

7) Is it valid to assume that the biochemical expressions of ischemia associated with angina pectoris are identical with those of the periinfarct zone of ischemia?

8) In the absence of generalized hypoxia, shock, and anemia will the administration of oxygen prove advantageous or detrimental to survival of ischemic myocardium?

9) Do lysosomal hydrolases initiate the terminal processes of ischemic myocardial cell death?

10) What is responsible for the "second wind" phenomena often seen with angina pectoris? Is there delayed vasodilation of precapillary sphincters? If so, how are they controlled?

11) What is the mechanism of action of experimental metabolic interventions (hyperosmolal mannitol, G.I.K., hyaluronidase, hydrocortisone, methylprednisolone, allopurinol, oxaloacetate + α-oxoglurate, fumarate + malate + glutamate)?

12) What objective measurements can we devise to determine the efficacy of a therapeutic intervention?

13) What is responsible for the early failure of contractility in the ischemic myocardium often seen in the presence of adequate high

energy phosphate stores? Is it due to compartmentation of ATP or to competition for membrane binding sites?

REFERENCES

1. Jennings, R. B.: Symposium on the pre-hospital phase of acute myocardial infarction. Part II: Early phase of myocardial ischemic injury and infarction. *Amer. J. Cardiol. 24:*753, 1969.
2. Brachfeld, N.: Maintenance of cell viability. *Circulation* (Suppl.) *39, 40:* iv-202, 1969.
3. Neely, J. R., Rovetto, M. J., and Oram, J. F.: Myocardial utilization of carbohydrate and lipids. *Progr. Cardiov. Dis. 15:*289, 1972.
4. Scheuer, J.: Myocardial metabolism in cardiac hypoxia. *Amer. J. Cardiol. 19:* 385, 1967.
5. Katz, A. M.: Effects of interrupted coronary flow upon myocardial metabolism and contractility. *Progr. Cardiov. Dis. 10:*450, 1968.
6. Opie, L. H.: Metabolism of the heart in health and disease. *Amer. Heart J. 76:*685, 1968.
7. Jennings, R. B., Kaltenbach, J. P., Sommers, H. M., Bahr, G. F., and Wartman, W. B.: Studies of the Dying Myocardial Cell. In: *The Etiology of Myocardial Infarction,* James, T. N. and Keyes, J. W., editors. Boston, Brown, 1963, chap. 12.
8. Brachfeld, N.: Myocardial Metabolic Dysfunction Following Infarction. In: *New Perspectives in Diagnosis and Management of Myocardial Infarction,* Corday, E. and Swann, H. J. C., editors. Baltimore, Williams & Wilkins, 1973, pp. 26-38.
9. Williamson, J. R.: Glycolytic control mechanisms: II. Kinetics of intermediate changes during the aerobic-anoxic transition in perfused rat heart. *J. Biol. Chem. 241:*5026, 1966.
10. Wollenberger, A. and Krause, E. G.: Metabolic control characteristics of the acutely ischemic myocardium. *Amer. J. Cardiol. 22:*349, 1968.
11. Morgan, H. E., Henderson, M. J., Regen, D. M., and Park, C. R.: Regulation of glucose uptake in muscle: I. The effects of insulin and anoxia on glucose transport and phosphorylation in the isolated perfused heart of normal rats. *J. Biol. Chem. 236:*253, 1961.
12. Brachfeld, N. and Scheuer, J.: Metabolism of glucose by the ischemic dog heart. *Amer. J. Physiol. 212:*603, 1967.
13. Neely, J. R., Whitfield, C. F., and Morgan, H. E. Regulation of glycogenolysis in hearts: Effects of pressure development, glucose and FFA. *Amer. J. Physiol. 219:*1083, 1970.
14. Danforth, W. H.: Activation of Glycolytic Pathways in Muscle. In: *Control of Energy Metabolism,* Chance, B., Estabrook, R. W., and Williamson, J. R., editors. New York, Acad. Press, 1965, p. 287.
15. Cornblath, M., Randle, P. J., Parmeggiani, A., and Morgan, H. E.: Effects of glucagon and anoxia on lactate production, glycogen content and phosphorylase activity in the perfused isolated rat heart. *J. Biol. Chem. 238:*1592, 1963.
16. Yang, W. C.: Anaerobic functional activity of isolated rabbit atria. *Amer. J. Physiol. 205:*781, 1963.
17. Weissler, A. M., Kruger, F. A., Baba, N., Scarpelli, D. G., Leighton, R. F., and Gallimore, J. K.: Role of anaerobic metabolism in the preservation of functional capacity and structure of anoxic myocardium, *J. Clin. Invest. 47:*403, 1968.
18. Wiliamson, J. R.: Personal communication.
19. Most, A. S., Gorlin, R., and Soeldner, J. S.: Glucose extraction by human myocardium during pacing stress. *Circulation 45:*92, 1972.
20. Opie, L. H., Thomas, M., Own, P., and Shulman, G. Increased coronary venous inorganic phosphate concentrations dur-

ing experimental myocardial ischemia. *Amer. J. Cardiol. 30:*503, 1972.

21. Parker, J. O., Chiong, M. A., West, R. O., and Case, R. B.: The effect of ischemia and alterations of heart rate on myocardial potassium balance in man. *Circulation 42:*205, 1970.
22. Opie, L. H. and Mansford, K. R. L.: The value of lactate and pyruvate measurements in the assessment of the redox state of free nicotinamide-adenine dinucleotide in the cytoplasm of perfused rat heart. *J. Clin. Invest. 1:*295, 1971.
23. Gorlin, R.: Assessment of hypoxia in the human heart. *Cardiology 57:*24, 1972.
24. Henderson, A. H., Craig, R. J., Gorlin, R., and Sonnenblick, E. H.: Lactate and pyruvate kinetics in isolated perfused rat hearts. *Amer. J. Physiol. 217:*1752, 1969.
25. Scheuer, J. and Berry, M. N.: Effect of alkalosis on glycolysis in the isolated rat heart. *Amer. J. Physiol. 213:*1143, 1967.
26. Huckabee, W. E.: Relationships of pyruvate and lactate during anaerobic metabolism. I. Effects of infusion of pyruvate or glucose and of hyperventilation. *J. Clin. Invest. 37:*244, 1958.
27. Neely, J. R., Denton, R. M., England, P. J., and Randle, P. J.: The effects of increased heart work on the tricarboxylic acid cycle and its interactions with glycolysis in the perfused rat heart. *Biochem. J. 128:*147, 1972.
28. Kuhn, P. and Pachinger, O.: The effect of calcium on myocardial lactate production under aerobic conditions. *J. Molec. Cell. Cardiol. 4:*171, 1972.
29. Rovetto, M. J., Whitmer, J. T., and Neely, J. R.: Comparison of the effects of anoxia and whole heart ischemia on carbohydrate utilization in isolated, working rat hearts. *Circ. Res. 32:* 699, 1973.
30. Bing, O. H. L., Brooks, W. W., and Messer, J. V.: Heart muscle viability following hypoxia: Protective effect of acidosis. *Science 180:*1299, 1973.
31. Leaf, A.: Regulation of intracellular fluid volume and disease. *Amer. J. Med. 49:*291, 1970.
32. Willerson, J. T., Powell, W. P., Guiney, T. E., Stark, J. J., Sanders, C. A., and Leaf, A.: Improvement in myocardial function and coronary blood flow in ischemic myocardium after mannitol. *J. Clin. Invest. 51:*2989, 1972.
33. Smithen, C., Keller, N., Christodoulou, J., and Brachfeld, N.: Metabolic and hemodynamic effects of hyperosmolal solutions on recovery from myocardial anoxia. *Clin. Res. 21:*451, 1973.
34. Wildenthal, K., Skelton, C. L., and Coleman, H. N., III: Cardiac muscle mechanics in hyperosmotic solutions. *Amer. J. Physiol. 217:* 302, 1969.
35. Selye, H.: *Chemical Prevention of Cardiac Necrosis.* New York, Ronald, 1959.
36. Sodi-Pallares, D., Bisteni, A., Medrano, G. A., DeMichili, A., Ponce De Leon, J., Calva, E., Fishleder, B. L., Testelli, M. R., and Miller, B. I.: The Polarizing Treatment in Cardiovascular Conditions. Experimental Basis and Clinical Applications. In: *Electrolytes in Cardiovascular Disease,* Bajusz, E., editor, Basel, Karger, 1966, p. 198.
37. Larcan, A.: Pathopsysiological Basis and Practical Application of a Metabolic Therapy of Myocardial Infarction. In: *Electrolytes in Cardiovascular Disease,* Bajusz, E., editor. Basel, Karger, 1966, p. 277.
38. Medical Research Council Working Party on the Treatment of Myocardial Infarction: Potassium, glucose and insulin treatment for acute myocardial infarction. *Lancet 2:*1355, 1968.
39. Mittra, B.: Potassium, glucose and insulin in treatment of myocardial infarction. *Brit. Heart J. 29:*616, 1967.
40. Maroko, P. R., Libby, P., Sobel, B. E., Bloor, C. M., Sybers, H. D., Shell, W. E., Covell, J. W., and Braunwald, E.: Effect of glucose-insulin-potassium infusion on myocardial infarction following experimental coronary artery occlusion. *Circulation 45:*1160, 1972.
41. Regan, T. J., Harman, M. A., Lehan, P. H., Burke, W. M., and Oldewurtel, H. A.: Ventricular arrythmias and K+ transfer during myocardial ischemia and intervention with procaine amide,

insulin or glucose solution. *J. Clin. Invest. 46*:1657, 1967.

42. Levinson, R. S., McIlduff, J. B., and Regan, T. J.: Comparison of polarizing solutions and isovolumic KCl in digitalis induced ventricular tachycardia. *Amer. Heart J. 80*:70, 1970.
43. Opie, L. H., Mansford, K. R. L., and Owen, P.: Effects of increased heart work on glycolysis and adenine nucleotides in the perfused heart of normal and diabetic rats. *Biochem. J. 124*:475, 1971.
44. Opie, L. H.: Substrate utilization and glycolysis in the heart. *Cardiology 56*:2, 1971-1972.
45. Scheuer, J. and Stezoski, S.: Protective role of increased myocardial glycogen stores in cardiac anoxia in the rat. *Circ. Res. 27*:835, 1970.
46. Hewitt, R. L., Lolley, D. M., Adrouny, G. A., and Drapanas, T.: Protective effect of myocardial glycogen on cardiac function during anoxia. *Surgery 73*:444, 1973.
47. Brachfeld, N., Ohtake, Y., Klein, I., and Kawade, M.: Substrate preference and metabolic activity of the aerobic and the hypoxic turtle heart. *Circ. Res. 31*:453, 1972.
48. Robin, E. D., Vester, J. W., Murdaugh, V., and Millen, J. E.: Prolonged anaerobiosis in a vertebrate: Anaerobic metabolism in the freshwater turtle. *J. Cell. Comp. Physiol. 63*:287, 1964.
49. Most, A. S., Brachfeld, N., Gorlin, R., and Wahren, J.: Free fatty acid metabolism of the human heart at rest. *J. Clin. Invest. 48*:1177, 1969.
50. Henderson, A. H., Most, A. S., and Sonnenblick, E. H.: Depression of contractility in rat heart muscle by free fatty acids during hypoxia. *Lancet 2*: 825, 1969.
51. Kjekshus, J. H. and Mjos, O. D.: Effect of free fatty acids on myocardial function and metabolism in the ischemic dog heart. *J. Clin. Invest. 51*:1767, 1972.
52. Oliver, M. F., Kurein, V. A., and Greenwood, T. W.: Relation between serum free fatty acids and arrythmias after acute myocardial infarction. *Lancet 1*:710, 1968.
53. Regan, T. J., Markov, A., Oldewurtel, H. A., and Burke, W. M.: Myocardial metabolism and function during ischemia: Response to l-noradrenaline. *Cardiov. Res. 4*:344, 1970.
54. Opie, L. H.: The general and local metabolic response to acute myocardial infarction. *Acta. Biol. Med. German. 28*:873, 1972.
55. Apstein, C. S., Gmeiner, R., and Brachfeld, N.: Effect of Palmitate on Hypoxic Myocardial Metabolism and Contractility. In: *Myocardiology*, Bajusz, E. and Rona, G., editors. Baltimore, London, Tokyo, University Park Press, 1972, p. 126.
56. Scheuer, J. and Brachfeld, N.: Myocardial uptake and fractional distribution of palmitate-1-^{14}C by the ischemic dog heart. *Metabolism 15*:945, 1966.
57. Wood, J. M., Hutchings, A. E., and Brachfeld, N.: Lipid metabolism in myocardial cell free homogenates. *J. Molec. Cell. Cardiol. 4*:97, 1972.
58. Brachfeld, N., Keller, N., Tarjan, E., Kline, S. A., and Apstein, C.: Myocardial metabolism following pacing induced stress. *Circulation* (Suppl.) *44*: 145, 1971.
59. Penney, D. G. and Cascarano, J.: Anaerobic rat heart: Effects of glucose and tricarboxylic acid-cycle metabolites on metabolism and physiological performance. *Biochem. J. 118*:221, 1970.
60. DeWall, R. A., Vasco, K. A., Stanley, E. L., and Kezdi, P.: Responses of the ischemic myocardium to allopurinol. *Amer. Heart J. 82*:362, 1971.